EARLY MANIFESTATIONS AND MECHANISM OF THE NEUROTOXIC ACTION OF ORGANOPHOSPHORUS PESTICIDES

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Since the 1930s, when about 20,000 cases of paralysis in man as a result of exposure to triorthocresyl phosphate (TOCP) were described, the problem of late neurotoxicity of organophosphorus compounds (OPC) has continued to interest research workers [3, 6, 7, 10, 13, 14]. However, the mechanism of onset and development of neuropathies under the influence of OPC has not been adequately studied. An important pathogenetic stage in the pathological process is phosphorylation of a protein specific for the nervous system, known as neurotoxic esterase (NTE) [8, 11]. NTE activity can be detected after selective inhibition of other esterases, which are unconnected with neurotoxicity [8]. The role of nonspecific esterases in the manifestation of neurotoxicity is not clear. The subsequent stages and manifestations of neuropathies, as far as the appearance of demyelination of motor axons, likewise have not been adequately investigated. Different views are held on the pathogenesis of these neuropathies [3]. In the present investigation, in order to study early manifestations and the mechanism of development of neurotoxicity, several compounds of a pesticide nature were used: trichlorphon, hostquick, etafos, mecarbam, etc. For comparison, a carbamic acid derivative, carbofuran was investigated. As standard reference compounds, for which the presence of a late neurotoxic effect in man had previously been established, TOCP and leptophos were chosen.

Birds and, in particular, hens [10], are known to be a suitable model for the study of late neurotoxicity. Since pareses and paralyses caused by exposure to OPC appear 2-4 weeks after their intake by birds, it was important to investigate changes arising in the latent period before develoment of clinical manifestations of neuropathy. The effect of OPC on acetylcholinesterase (AChE) activity in different parts of the nervous system and on cholinesterase (ChE) activity in the blood of hens was studied. The effect of OPC on NTE activity was investigated in experiments in vitro and in vivo. OPC with and without the property of inducing a late neurotoxic action were chosen, with the aim of determining the value of investigating the activity of this enzyme in order to predict the late neurotoxic effect in model experiments. To determine the time course of progression of the neuropathy, the velocity of spread of excitation (VSE) along the hens' peripheral nerves was determined in the latent period and at different times of the experiment.

EXPERIMENTAL METHODS

Experiments were carried out on 84 White Russian hens weighing 1.5-2 kg. The functional state of their peripheral nervous system was assessed by measuring VSE in a branch of the peroneal nerve innervating the abductor digiti II muscle of the hen's lower limb. VSE was recorded at intervals over a period of time in the same birds without any operative intervention [4]. The experimental groups contained six-eight birds. OPC, over a wide dose range, were introduced into the crop, in a single dose, in the form of aqueous emulsions. AChE and ChE activity in various biological substrates and NTE activity in the brain and spinal cord after exposure to OPC were determined periodically by the methods in [9] and [12] respectively. In the experiments in vitro, NTE activity of hen brain homogenates was determined after the use of pesticides in concentrations of 10^{-2} to 10^{-5} M. Inhibition of the enzyme

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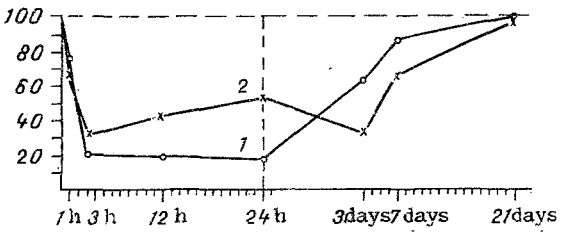

Fig. 1. Inhibition of NTE in hen brain (1) and spinal cord (2) by mecarbam in a dose of 200 mg/kg. Abscissa, time after administration of pesticide; ordinate, inhibition of enzyme (in %).

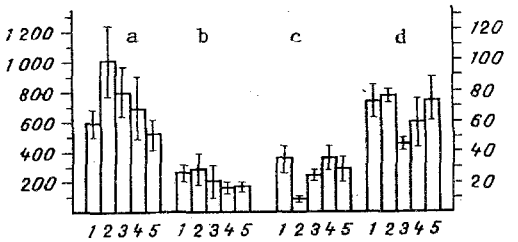

Fig. 2. ChE and AChE activity in biological substrates from hens after administration of mecarbam in a dose of 200 mg/kg. Ordinate, enzyme activity: on left – in mmoles/h/liter blood serum, on right – in mmoles/h/kg tissue. a) AChE in cerebral hemispheres; b) AChE in spinal cord; c) ChE in blood serum; d) ChE in sciatic nerve. 1) Control; 2-5) enzyme activity 1, 7, 14, and 21 days respectively after administration.

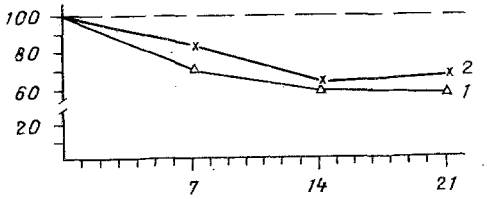

Fig. 3. Changes in VSE along hens' peripheral nerve after administration of TOCP in a dose of 1 g/kg (1) and of mecarbam in a dose of 200 mg/kg (2). Abscissa, time after administration of compounds (in days); ordinate, VSE (in % of control).

was expressed as a percentage of the control. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The hens developed ataxia, pareses, and paralyses on the 14th-21st day after administration of TOCP in doses of 0.8-1.2 g/kg, of leptophos in doses of 1-3 mg/kg, and of mecarbam in doses of 50-200 mg/kg.

Trichlorphon, DDVP, etafos, hostaquick, and also carbofuran, in toxic doses (LD_{50} or more) caused no late neurotoxic effect.

On incubation of brain homogenates with OPC possessing neurotoxic properties, NTE activity was found to be depressed in the experiments with TOCP by 88%, with leptophos by 63%, with mecarbam by 69%, and with its oxyphosphonate metabolite by 88%. Meanwhile etafos, carbofuran, and hostaquick, when used in the same concentration (10^{-3} M), inhibited NTE much less strongly: etafos by 14%, carbofuran by 30%, and hostaquick by 30%. TOCP and mecarbam caused marked (by more than 50%) inhibition of NTE even in concentration of 10^{-4}-10^{-5} M.

Correlation was thus found between the neurotoxic action of OPC and their ability to inhibit NTE activity in vitro.

The effect of OPC on NTE activity in experiments in vivo was studied in relation to mecarbam, which has a selective and delayed neurotoxic action over a wide dose range – from 3000 mg/kg (LD_{50}) to 25 mg/kg (1/120 LD_{50}), without producing any clinical manifestations of poisoning.

Only 1 h after administration of mecarbam in a dose of 200 mg/kg, NTE activity in the brain and spinal cord was inhibited (Fig. 1). Maximal inhibition of NTE was recorded 3-24 h after administration of the pesticide. This was followed by a tendency toward recovery of enzyme activity. However, it had not regained its initial value after 7 days. On the 21st day, when lasting paralyses were observed, NTE activity was the same as in the control. Significant (by 50%) inhibition of NTE in the brain and spinal cord also was observed 24 h after administration of mecarbam in a dose of 50 mg/kg.

Mecarbam has weak anticholinesterase activity, but in toxic doses it inhibits ChE in the blood of albino rats [1]. It was interesting to study the time course of changes in ChE and AChE activity in the blood serum and brain of hens. After administration of mecarbam in a dose of 200 mg/kg, significant inhibition of ChE activity in the blood serum took place on the 1st day of the experiment, followed by recovery on the 14th day (Fig. 2). On the 7th day ChE activity of the sciatic nerve was inhibited (by 40%), and returned to normal by the 21st day. Incidentally, mecarbam, under these circumstances, did not inhibit AChE activity in different parts of the brain or spinal cord, and it was actually increased in the cerebral hemispheres and medulla and diencephalon. These data are evidence of the absence of correlation between inhibition of AChE in nerve tissue and neurotoxic action. At the same time, correlation was observed between the effect of mecarbam on the serum ChE level and on NTE. Since the serum ChE specifically binds OPC, its inhibition can evidently facilitate the passage of OPC through the blood-brain barrier, with inhibition of NTE.

The marked slowing of VSE observed during the latent period (7th day) and later against the background of clinically manifest neuropathy (14th-21st days) indicates loss of excitability primarily in thick (A-α) fast-conducting nerve fibers (Fig. 3). Under these circumstances the process of demyelination in peripheral nerves and in the spinal cord was confirmed morphologically. Consequently, the data obtained confirm that slowing of VSE is an early manifestation of late neurotoxicity, for it appears long before pareses and paralyses. Meanwhile slowing of VSE develops actually against the background of inhibition of NTE. NTE inhibition is the trigger mechanisms of the neuropathy, and slowing of VSE develops against the background of initial morphological and functional changes in myelin. The process progresses after a single dose of the neurotoxic agent, despite restoration of cholinesterase activity – a specific manifestation of the toxic action of OPC [2] and of activity of NTE, which is responsible for development of the neuropathy.

The experimental results thus confirm that the first stage in the development of the delayed neurotoxic action of OPC is inhibition of NTE, a specific nerve tissue protein. Inhibition of pseudocholinesterase is evidently a facilitating factor. To predict the ability of OPC to exert a late neurotoxic action, their effect on NTE activity can be investigated in vitro and in vivo. One early manifestation of neuropathy is slowing of VSE along the peripheral nerves of hens, which is observed before the development of other clinical changes (ataxia, pareses, paralyses).

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EFFECT OF CHRONIC ETHANOL INTAKE ON PERMEABILITY OF THE BLOOD–BRAIN BARRIER FOR ^{14}C-TYROSINE AND HORSERADISH PEROXIDASE IN RATS

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Administration of a single dose of ethanol has been shown to increase the permeability of the blood–brain barrier (BBB) for precursors of certain neurotransmitters and, in particular, for tryptophan [8] and dopa [3], possibly as a result of mobilization of adaptive processes aimed at making good the neurotransmitter deficiency [1] arising under the influence of ethanol. At the same time, there is evidence to show that chronic ethanol consumption by animals leads to inhibition of transport of the serotonin precursor, tryptophan, into the brain and this is accompanied by a fall in the neurotransmitter level in the brain [2]. This phenomenon may lie at the basis of changes in activity of the transport function of BBB under the influence of chronic ethanol intake. Morphological investigations [5] have shown that transendothelial pinocytosis probably arises during chronic ethanol intake by animals, possible evidence of changes in the barrier function of BBB.

The aim of this investigation was to study the effect of chronic alcohol administration to animals on the transport and barrier functions of BBB with respect to peripherally injected ^{14}C-tyrosine.

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar rats weighing 380-450 g, divided depending on the quantity of alcohol consumed during 3 weeks of free choice between 10% ethanol solution and water, into heavy drinkers (over 3.5 g ethanol/kg body weight daily) and light drinkers (under 2 g ethanol/kg body weight daily). After division of the animals in this way, a 25% solution of ethanol was administered by the intragastric route 3 times a day for 10 days in doses which increased daily (8-11 g/kg). Control animals received equivalent volumes of physiological saline by gastric tube. The experimental heavy and light drinking animals were divided into four groups: in the animals of group 1, the last (30th) dose of

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